Adapter-directed display systems

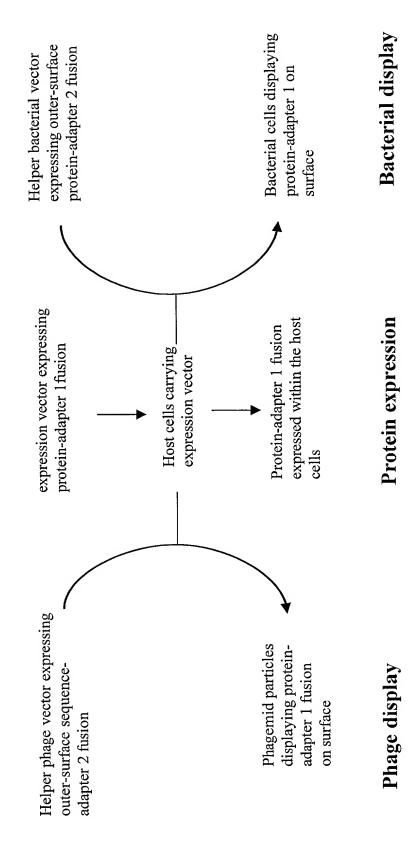
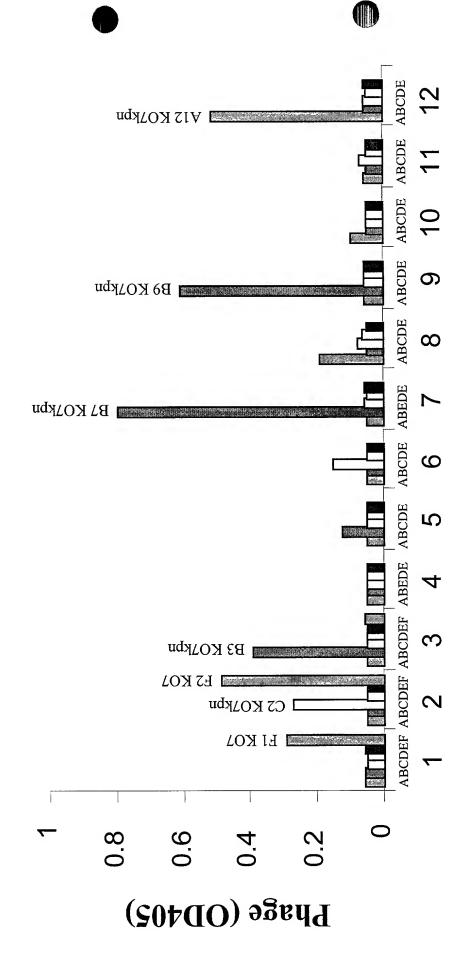


Fig. 1

KO7kpn phage Screening by ELISA



KO7kpn helper phage Vector

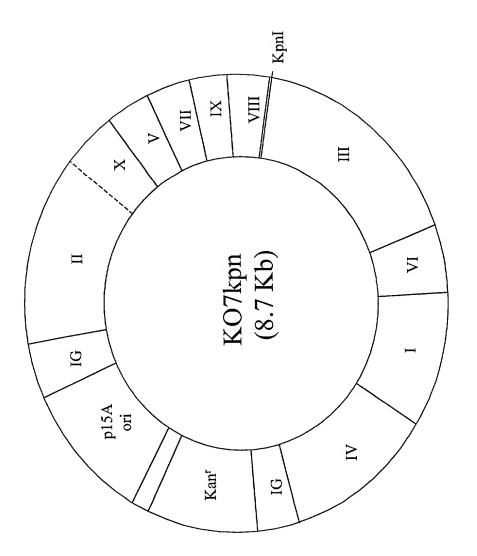


Fig. 3A

Gene III leader sequence in KO7 helper phage

GTG AAA AAA TTA TTC GCA ATT CCT TTA GTT GTT CCT TTC TAT TCT CAC TCC GCT V K K L L F A I P L V V P F Y S H S A

Gene III leader sequence in KO7kpn helper phage

GTG AAA AAA TTA TTC GCA ATT CCT TTA GTG GTA CCT TTC TAT TCT CAC TCC GCT V K K L L F A I P L V V P F Y S H S A Kpnl

Map of phagemid vector pABMC6

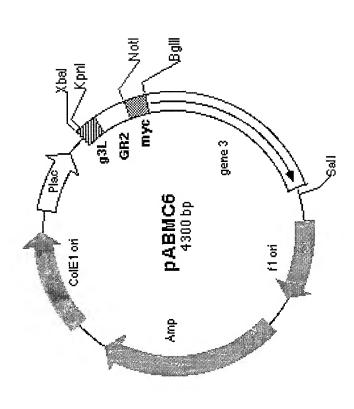


Fig. 4

Helper phage with engineered gene III fused to adaptor 2

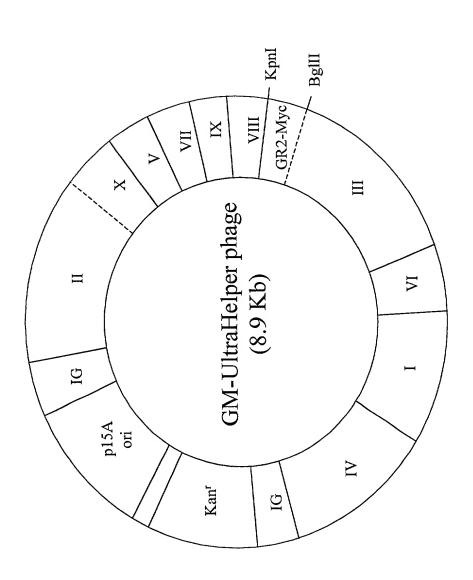


Fig. 5A

GR2-Myc domain coding sequence in GM-UltraHelper phage genome

---TTAGTGGTACCTTTCTATTCTCACTCCGCT ACATCCCGCCTGGAGGGCCTACAGTCAGAAAACCATCGCCTGCGA Ы 凶 Ø J U 闰 Н ĸ യ H Þ ß Ħ Gene III leader മ ¥ L V V P ATGAAGATCACAGAGCTGGATAAAGACTTGGAAGAGGTCACCATGCAGCTGCAGGACGTCGGAGGTTGC GCGGCCGCA A A A U ט D Q ø н α Σ H > 闰 团 Н Д ¥ L D 凶

NotI

GAACAAAAACTCTCAGAAGAGGATCTG AGATCTGGAGGCGGT ACTGTTGAAAGTTGTTTAGCAAAA---Ц บ മ Gene III T VE ט ט BgIII ъ В Ы Д 闭 Myc-tag മ П α

Trypsin cleavage sites at GR2-Myc domain on GM-UltraHelper phage

E T S R L E G L Q S E N H R L R M K I T E L D K D L E GR2 domain

TMQLQDVGGCAAAEQKLISEEDLRSGGG Myc-tag

Fig. 5C

GR2-Myc-pIII fusions assembly into GM phage particles

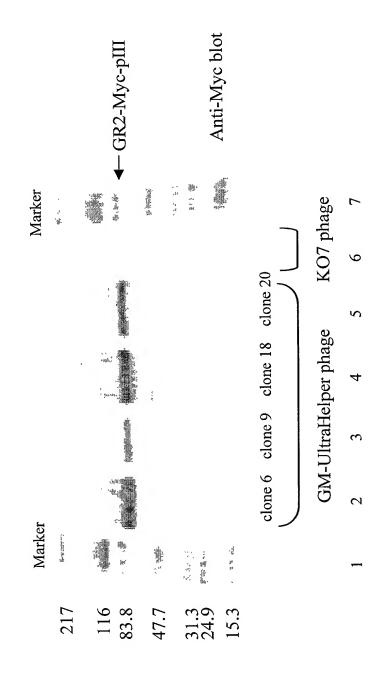
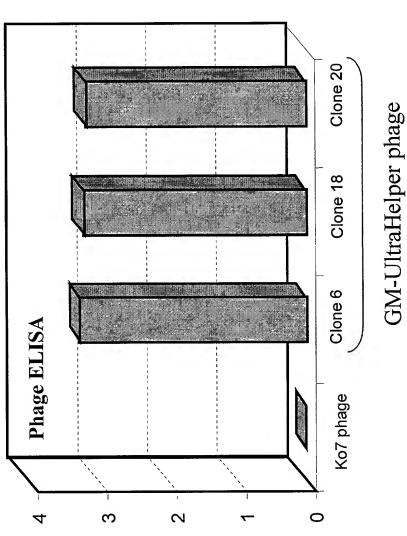


Fig. 6

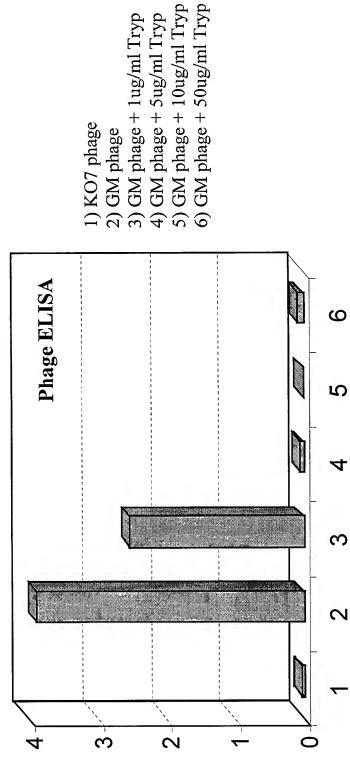
Detection of GR2-Myc domain on GM-UltraHelper phage



Phage binding to anti-Myc antibody

Fig. 7

Cleavage of GR2-Myc domains on GM phages by trypsin



Phage binding to anti-Myc antibody

Phagemid vector for protein-GR1 expression

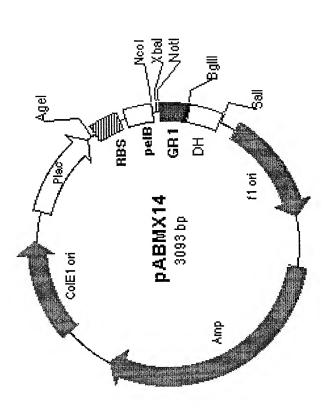


Fig. 9A

Complete vector sequence of pABMX14

GCCTTCCTGTTTTTGCTCACCCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTTCGC atctaggtgaagatcctttttgataatctcatgaccaaaatcccttaacgtgagttttcgttccactgagcgtcagaccccgtagaaaagatcaaaggatcttgagatcc ICCTICTAGIGIAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGIGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGG ITGGACTCAAGACGATAGTTACCGGGATAAGGCGCAGCGGTCGGACGGGGTTCGTGCACACACCCAGCTTGGAGCGAACGACCTACACCAGATACTGAGATACCTACAGCGTGAGCTATG GCGCAACGCAATTAATGTGAGTTAGGCTCACTCATTAGGCACCCCAGGCTTTTACACTTTCCGGCTCGTATGTTGTGTGGAATTGTGAGCGGATAACAATTTACCGGTTCTTAAGGAGGA AITAAAAAATGGAAATACCTATGCCTACGGCGGCGGCGGGGTTATTACTCGCGGCCCAGCCGGGCCATGGCGGCCCTGCAGGCCGTCTAGAGCGGCCGCTGGAGGTGAGGAAGTCCCGGCTG ITGGAGAAGGAGAACCGTGAAAAAAAAAATCATTGCTGAGAAAGGAGGGGGGTGTCTCTGAACTGCGCCATCAACTCCAGTCTTAGGAGGTTGTAGATCTTATCCATACGACGTACCAGACTA accaacttaatgecettgcagcactcccctttcgccagctggcgtaatagcgaagggcccgcaccgatcgcccttcccaacagttgcgcagcctgaatggcgaatgggacg CGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAAACTGGAACAACCACTCAACCTATCTCGGTCTATTCTTTTGAAGGGATTTTGCCGATTTTCGGCCTATTG ACAITICAAATATGTAITCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATTTGAAAAGGAAGAGTATGAGTATTCCAACAITTCCGTGTCGCCCTTATTCCCTTTTTGCGGCCAITTT CTGGTTTATTGCTGATAAATCTGGAGCCCGGTGAGCGTGCGCGGTATCATTGCAGCCACACTGGGGCCAGATGGTAAGCCCTCCCGTATGTTATCTACACGACGGGGAGTCAGGCAACTA AGAAAGCGCCACGTTCCCGAAGGGAGAAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGGAGCGACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTAATAGTCCTGTCCT CGCAGGAGGTCATCATCATCATCATTAATGAGTCGACCTCGACCAATTCGCCCTATAGTGAGTCGTATTACAATTCACTGGCCGTCGTTTTACAACGTCGTGGAACTGGGAAAACCCTGGCGTT CCCGAAGAACGTTTTCCAATGATGAACCACTTTTAAAGTTCTGCTATGTGGCGCGGGTATTATCCCGTATGACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAATGACTTGGT GTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTTCGGTTTTAGTGCTTTTACGGCACCTCCAAAAAACTTGATTAGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGATAGACGGTTTTTT SCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTCATTAATGCAGGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGGCAGTGA

Fig. 9B

scFv-GR1/GM-UltraHelper phage scFv-GR1/KO7 helper phage Functional display of scFv by GM-UltraHelper phage 10^{13} 10^{12} phage (particle/ml) 10^{11} 10^{10} 10^{9} 2 3 phage binding to antigen (OD405)

Fig. 10

Mutivalent display of scFv by GM-UltraHelper phage

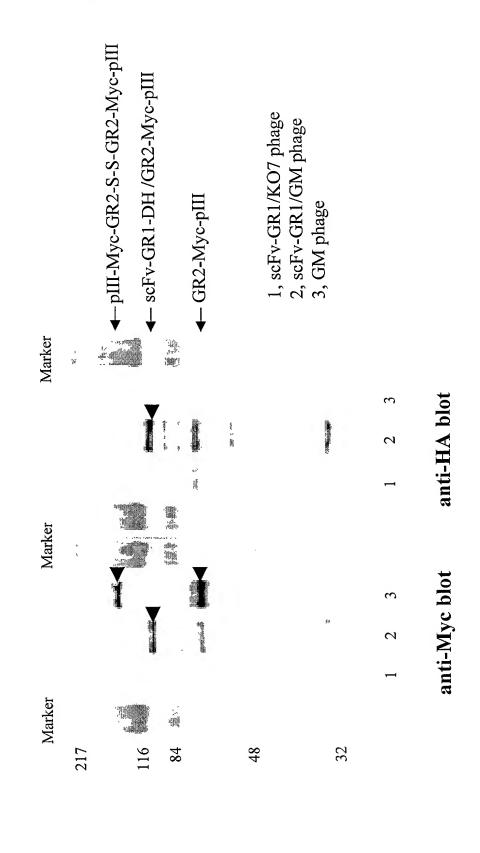


Fig. 11

Map of phagemid vector pABMC13

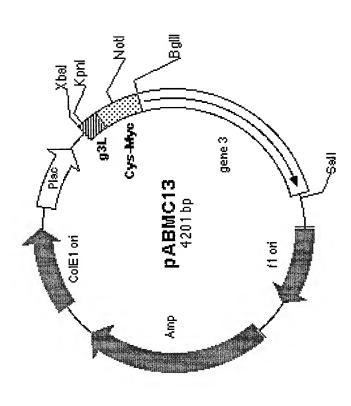


Fig. 12

Helper phage with Cys-Myc-pIII fusion gene

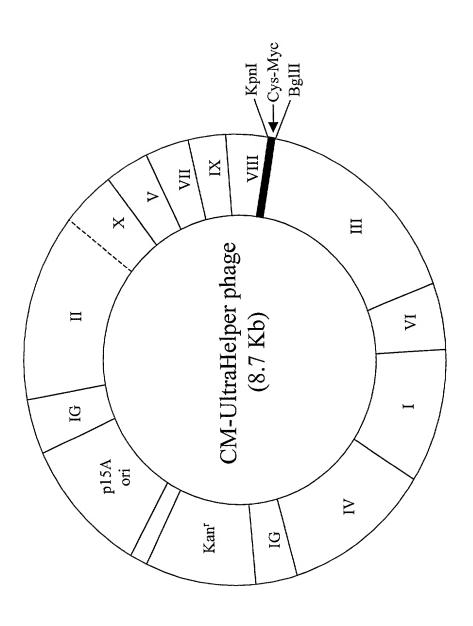


Fig. 13A

Engineered gene III sequence in CM phage

---TTAGT<u>GGTACC</u>TTTCTATTCTCACTCCGCT <u>TAG</u>GCTTGCGGTGGT<u>GCGGCCGC</u>AGAACAAAAACTCATCTCAGAAGAACTGAGATCT <u>AGAICT</u>GGA Myc-tag α 臼 G A A A NotI ტ KpnI Gene III leader Amber stop S A * A C гуургун

GGCGGT ACTGTTGAAAGTTGTTTAGCAAAACCTCATACAGAAATTCATTTACTAACGTCTGGAAAGACGACAAAACTTTAGATCGTTACGCT-----Ц ĸ Д Д X × > z H ĮΉ വ z 闰 H Ħ Д × LA ບ Ø 臼 Gene III D T ტ

Fig. 13B

Detection of Myc-tag on CM-UltraHelper phages by ELISA

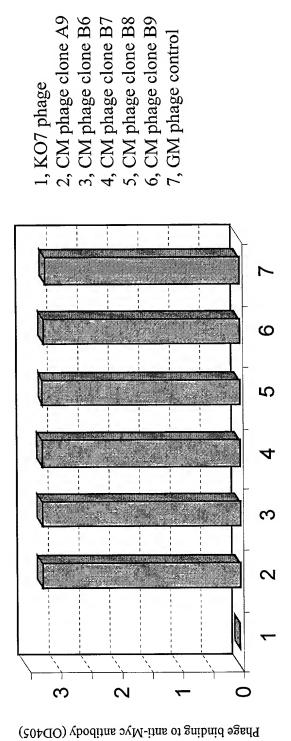


Fig. 14

Phagemid vector for protein-HA-cys expression

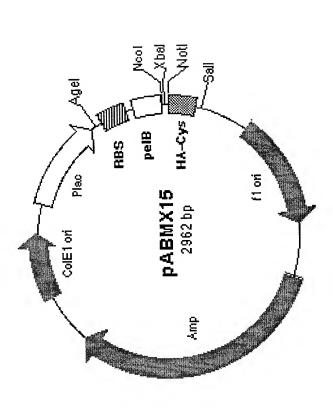


Fig. 15A

Complete vector sequence of pABMX15

GCACACAGCCCAGCTTGGAGCGAACGTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAA TCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAAGTTTACTCATATATACTTTAAATTGATTTAAAACTTCATTTTAATTTAAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCA CGGIGGITIGICGGGAICAAGAGCIACCAACICITITICCGAAGGIAACIGGCITCAGCAGAGCGCAGAIACCAAAIACIGICCITCTAGIGIAGCGGIAGITAGGCCACCACITCAAGAACICIGIAGCAC altaaaaaatgaaatacctatgcctacggcgctggattgttattactcgcggcccagccggccatgcggccttgcaggcctctaaaggcggccgcttacccgtacgacgttccggactacgcaggtggct GCTGATAAGTCGACCTCGAACTAGCCCTATAGTGAGTCGTATTACAATTCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTTC CTCGACCCCAAAAAACTTGATTAGGGTGATGGTTCACGTAGTGGGCCATCGGCCCTGATAGACGGTTTTTCGCCCTTTGACGTGCCACGTTCTTTAATAGTGGACTCTTGTTCCAAACTGGAACAACAACTC aaccctatctcggtctattcttttgaitttagaggaitttggccgatttgggttaaaaaatgagctgaittaacaaaaatttaacgaaatttaacaaatataacgtaggca CITITCGGGGAAATGTGCGCGGAACCCCTAITTGTTTTTTTTTAAATACAITCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAAC ALTICCGTGTCGCCTTATTCCCTTTTTTGCGGCATTTTGCTTTTTTGCTCACCCAGAAACGCTGGTGAAAGGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTTACATCGAACTGGATCTCA acagcggtragatccttgagagttttcgccccgaagaacgttttccaatgatgagcactttttaaagttctgctaigtggcggggatttatcccgtattgacgccgggcaagagcaactcggtcgccgcatacact AGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGGGCCCCAATACGGCAAACCGCCTCCCCCGCGCTTGGCCGATTCATTAATGCAGCTGGGACGACAGGTTTCCCGGAAAGCGGGGAAGTGA

Fig. 15B

Functional display of scFv by CM-UltraHelper phage

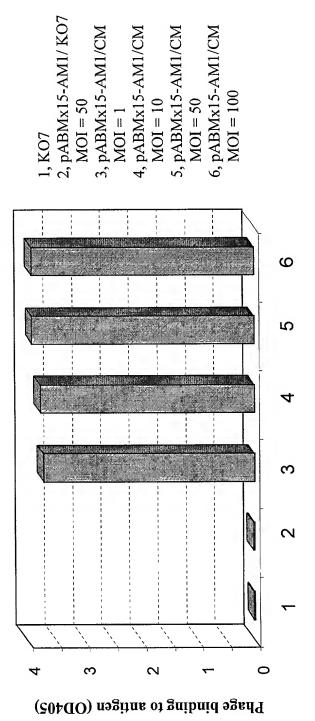
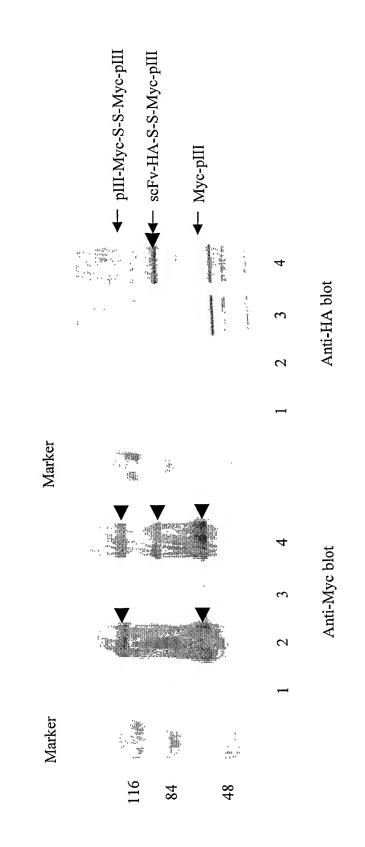


Fig. 16

Detection of scFv displayed by CM-UltraHelper phage



1: KO7 phage; 2: CM phage; 3: pABMx15-AM1/KO7; 4: pABMx15-AM1/CM

Fig. 17

Map of phagemid vector pABMC12

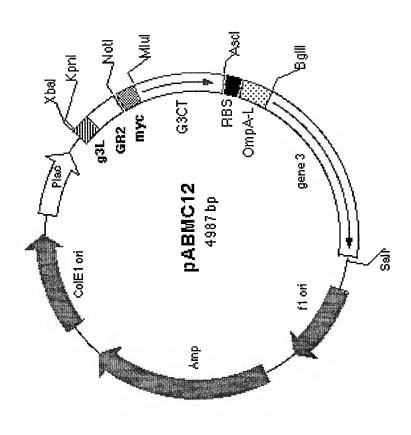


Fig. 18

Helper phage with an additional copy of engineered gene III

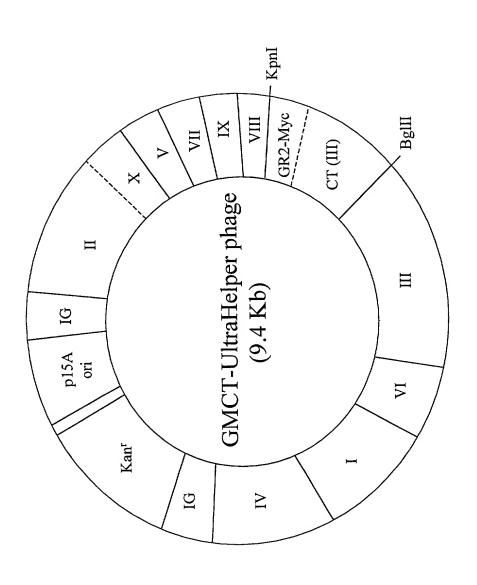


Fig. 19A

Engineered gene III Sequence in GMCT phage genome

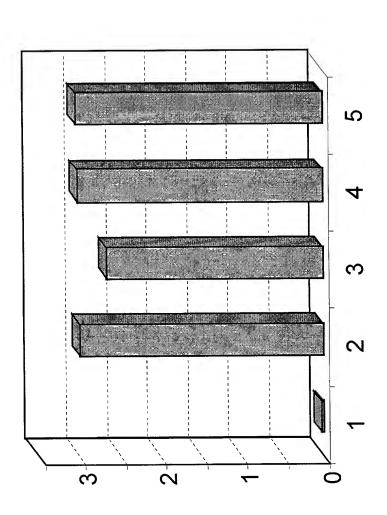
--TTAGT<u>GGTACC</u>TTTCTATTCTCACTCCGCT ACATCCCGCCTGGAGGGCCTACAGTCAGAAAACCATCGCCTGCGAATGAAGATCACAGAGCTGGATAAA GGTTCCGGTGATTTTGATTATGAAAAAATGGCAAACGCTAATAAGGGGGGCTATGACCGAAAATGCCGATGAAAACGCGCTACAGTCTGACGCTAAAGGCAAA CCTITITGTCTTTGGCGCTGGTAAACCATATGAATTTTTTTTTGTGACAAAATAAACTTATTCCGTGGTGTCTTTGCGTTTTTTTATATATGCCACC CTTGATTCTGTCGCTACTGATTACGGTGCTGCTATCGATGGTTTCATTGGTGACGTTTCCGGCCTTGCTAATGGTAATGGTGCTACTGGTGATTTTTGCTGGC GGCTCTGGTGGTTGTTGGTGGCGGCTCTGAGGGTGGCGGCTCTGAGGGTGGCGGTTCTGAGGGTGGCGGCTCTGAGGGTGGCGGTGGCGGTTCCGGTGGCGGCTGC GACTIGGAAGAGGICACCAIGCAGCIGCAGGACGICGGAGGIIGC <u>GCGGCCGC</u>AGAACAAAAACIGAICTCAGAAGAGAGAICIGACGCGIGCI GGCGGC > Н ø ტ × Д ט N A L Ħ S E N D 团 Myc-tag Д > ಬ Ü ט N A D E G L A N O Y L ద GR2 domain Н ט N N ט ы ы 田 P L M N N F R CDKINL F D Y E K M A N A N K G A M T E တ ಬ ы ט D V Ø A A A ש ᆟ Noti ט ტ F H G 闰 闰 ບ ß ט ഗ ט U N H æ ט ט Q Z Q D Ø ш ט Λ Q Q Н ď 闰 闰 Gene III Leader A T D Y G A D D × ß ᅜ ტ ц О Ö > CT domain of Gene III ტ ŋ M A Q T T ø r U ש O) D G ა |> ט ŋ

AAGACAGCTATTGCGATTGCAGTGGCACTGGCTGGTTTCGCTACCGTAGCGCAGGCT <u>AGATCT</u>GGAGGCGGT ACTGTTGAAAGTTGTTTAGCAAAA---บ Gene III > ט Ü Bglii ß ഷ ⋖ ο വ Þ > 闰 × ₽ z ద Н Ö н ď z Ы Ø Ľτ E ď ß ď ш >

ttratgtatgtattttctacgtttgctaacatactgcgtaataaggagtcttaataa <u>ggcgccc</u>acaatttcacag<u>taaggagg</u>tttaataa atgaaa

Fig. 19B

Functional display of scFv by GMCT-UltraHelper phage



1, pABMx14-AM1/ KO7 MOI = 50

2, pABMx14-AM1/GMCT MOI = 1 3, pABMx14-AM1/GMCT

3, pABMx14-AM1/GMCT MOI = 10 4, pABMx15-AM1/GMCT MOI = 50

5, pABMx14-AM1/GMCT MOI = 100

Fig. 20

Detection of scFv displayed by GMCT-UltraHelper phage

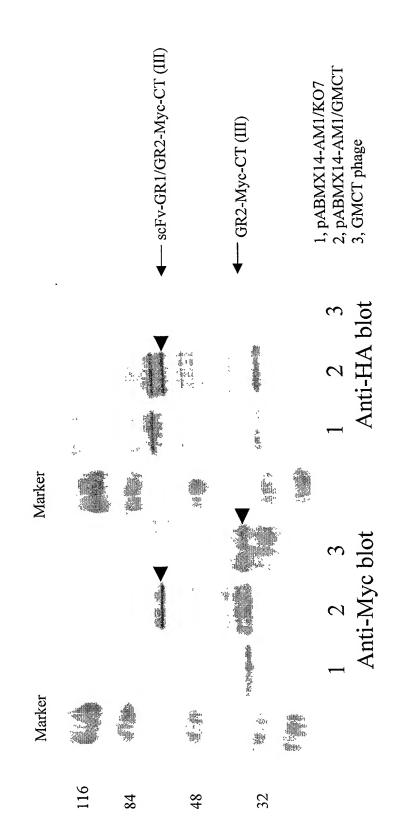


Fig. 2]

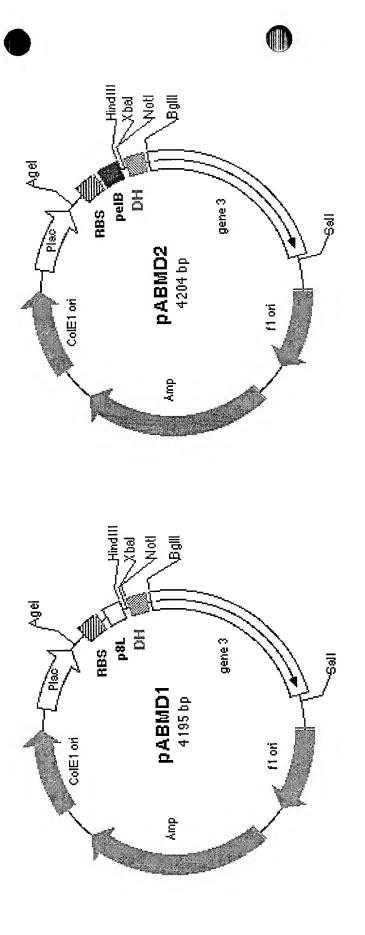


Fig. 22A

PABMD1 vector: sequence from Agel to Sall

GGAGGCGGT ACTGTTGAAAGTTGTTTAGCAAAA ---- GCTAACATACTGCGTAATAAGGAGTCTTAA GTCGAC AGATCT ATGAAAAAGTCTTTAGTCCTCAAAGCCTCCGTAGCCGTTGCTACCCTCGTTCCGATGCT<u>AAGCTT</u>CGCT <u>ICTAGA</u> Sall Amber stop BgIII HindIII AATTGTGAGCGGATAACAATTT ACCGGT TCTT TTAACTTTAG TAAGGAGG AATTAAAAA GCGGCCGCT TATCCATACGACGTACCAGACTACGCA GGAGGT CATCACCATCATCACCAT SVAVATLVPMLS н н н н н Z His-tag S/D <u>ტ</u> Y Y P Y D V P D Gene 3 SLVLKA HA-tag Ø P8 Leader lac promoter/lac O1

PABMD2 vector: sequence from Agel to Sall

ATGAAATACCTATTGCCTACGGCAGCCGCTGGATTGTTATTACTCGCGGCCCAGCCGG<u>CCATGG</u>CGGCC<u>CTGCAG</u>GCCT<u>GCAG</u>GCCTTACTAAAA Xbal AGATCT GGAGGCGGT ACTGTTGAAAGTTGTTTAGCAAAA ---- GCTAACATACTGCGTAATAAGGAGTCTTAA <u>GTCGAC</u> Amber stop BgIII LLPTAAGLLLLAAQPAMAAL AATTGTGAGCGGATAACAATTT ACCGGT TCTT TTAACTTTAG TAAGGAGG AATTAAAAA GCGGCCGCT TATCCATACGACGTACCAGACTACGCA GGAGGT CATCACCATCATCACCAT Nco I 闰 ннннн His-tag S/D ტ EP 1 1 Y P Y D V P D Gene 3 HA-tag ט ഗ pelB Leader ſΊ lac promoter/lac O1 Ü NotI Ü

Fig. 22B

GR1 Sequence Range: 1 to 146

GR2 Sequence Range: 1 to 140

Fig. 23

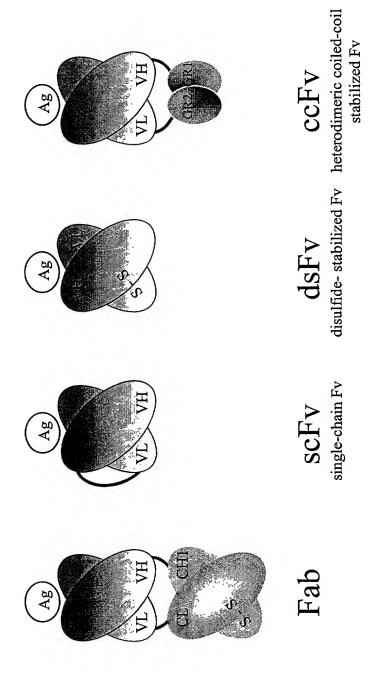


Fig. 24

antigen

Ag

Expression vector for Adapter-directed bacterial display

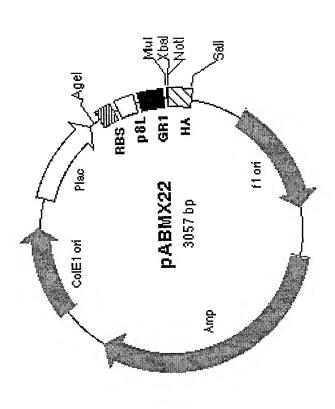


Fig. 25A

Complete vector sequence of pABMX22

GACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTTTCTCAGAATGACTTGGTTGAGTACTCACCAGTAAAAAGCATCTTACGGATGGCATGACAGTAAGAATTATGCAGTGCTGCCAT tactitagattgaatttaaaacttcattttaatttaaaaggatctaggtgaagatccttttttgataatctcatgaccaaaatcccttaacgtgagttttcgttccactgagcgtcagaccccgtagaa aactggcttcagcagagcgcagataccaaatactgtccttctagtggtagttaggccaccacttcaagaactctgtagcaccgcctacatacctcggtctgatcctgttaccagtggctg gaactgagatacctacagcgtgagctatgagaaagcgccacgcttcccgaagggagaaaggcggacaggtatccggtaagcggcagggtcggaacaggaggcgcacgagggagcttccagggggaaa attgctgagaaagaggagggtgttctgaactgcgactacagtctgtaggcggttgcacgcgttctagagcggccgcttacccgtacgacgtccggactacgcatgataagtcgacctcga ACTIGCCAGCGCCCTAGCGCCCCTTTCCTTTCCTTTCTTCCTTTCTCGCCACGTTTTCCCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTTTACGGC aaggaagatatgagtattcaacatttccgtgtcgcccttattttgcggcattttgcccttcctcttttgcggtaaaagtaaaagtaaaagtaaaagatgaagatcagttg aaccatgagtgataacactgcggccaacttacttctgacaacgatcggaggaccgaaggagctaaccgcttttttgcacaacatggggggatcatgtaactcgccttgatcgttgggaaccggagctga CTGCCAGTGGCGATAAGTCGTGTTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGGGTTCGAACGGGGGGGTTCGTGCACACCCAGCTTGGAGCGAACGACCTACACC aaaaaatgaaaaagtctttagtcctcaaagcctccgtagccgtgctaccctcgttccgatgctacgttcgctggtgagaaagtcccgtctgctggagaaagagaaaccgtaaaaaga ccaattcgccctatagtgagtcgtattacaattcactggccgtcgttttacaacgtcgtgactgggaaaaccctggcgttacccaacttaatcgccttgcagcactttcgccagctggcgt acaacactcaaccctatctctgtttttttttttaattttgccgattttcgcctattggttaaaaatgagctgatttaacaaaatttaacgaaatttaacaaattttaacg GTGAGCGAGGAAGGGGGAAGAGCCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTCATTAATGCAGCTGGCACGACAGGTTTCCCCGACTGGAAAGCGGGCAGTGA

Fig. 25B

Helper vector for adapter-directed bacterial display

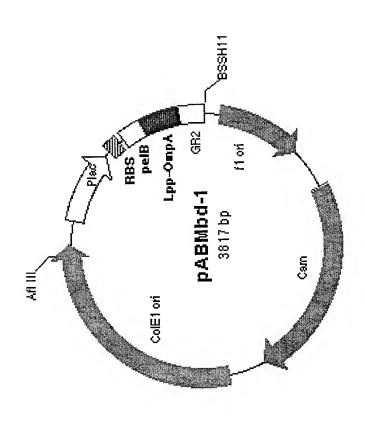


Fig. 26A

Complete vector sequence of pABMXbd-1

actetgacttgagegtegatttttgtgatgetegteggeggeggeggeetatggaaaaaegeegeeagegeeetttttaeggetteetggeettttgetegeetttteet IGAGAATATGTTTTCGTCTCAGGCAATCCCTGGGTGAGTTTCACCAGTTTTGATTTAAACGTGGCCAATATGGACAACTTCTTCGCCCCGTTTTCACCATGGGCAAATATTATACGCAAGGCGACAAG ttctgcgcgtaatctgctgctaarcaaaaaaacaccgctaccagcggtggtttgtttgccggatcaagagctaccaactctttttccgaaggtaactggcttcagcagagcgcagataccaaata CGTIGATATECCCAATGGCATCGTAAAGAACATTTTGAGGCATTTCAGTTGGTTCAATGTACCTATAACCAGACCGTTCAGCTGGATATTACGGCCTTTTTAAAGACCGTAAAGAAAAATAAGCA CAAGITITATCCGGCCITTATTCACAITCTTGCCCGCCTGATGATGCTCATCCGGAAITACGTAIGAAAGACGGTGAGGTGGTGATATGGGATAGTGTTCACCCTTGTTACACCGTTTTCCA tgagcaaactgaaacgttttcatcgctctggagtgaataccacgacgatttccggcagtttctacacatatattcgcaagatgtgggggtgttacggtgaaaacctggcctatttccctaaagggtttat tattggtgcccttaaacgcctggttgctacgcctgaataagtgataataagcggatgaatggcagaaattcgaaaattcgacccggtcgttcaggggcagggtcgttaaataggccgcttatg gatcaaaaggaictaggtgaagaicctittigataatctcaigaccaaaaicccttaacgtgagtittcgttccactgagcgtcagaccccgtagaaagatcaaaggatcttcttgagatctttt CTGTCCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCAGTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTTTACCGGGT IGGACTCAAGACGATAAGTTAAGGCGCAGCGGGTCGGGCTGAACGGGGGGGTTCGTGCACAGCCCAGCTTGGAGCGAACGACCTACACGGAATGGATACCTACAGCGTGAGCTATGAGAAA GCGCCACGCTTCCCGAAGGGAAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCC tgaitacgccaagcgcgttaactttagtaaggaggaattaaaaaatgaaatgctgctgccgagcggggtttgctgttactggcggcccagccggctatggcgatgaaagctactaaactg gtactgggcaacccgtatgttggctttgaaatgggttacgactggttaggtcgtataaaaggcagcgttgaaaacggtgcatacaaagctcaggggcgttcaactgaccgctaaactgggttac ccaatcactgacctggacatctacactcggctggctaggtatggcgtgccagacactaaatccaacgtttatggtaaaaaccacgacatttctccggtcttcgctggcgctgtgag CATCGCCTGCGAATGAAGATCACAGAGCTGGATAAAGACTTGGAAGATCACCATGCAGCTGCAAGACGTTGGCGGTTGCTAATGAGCGCGCTCACTGGCCGTTTTTACAACGTCGTGACTGGGAA aaccetgegettacecaacttaategecettgeageacateeecetttegeceagetggegtaatagegaagggeegeeecegategecettgeegegegetggegaatggeaatggeaegege CCCCGICAAGCICIAAAICGGGGGCICCCITIAGGGIICCGAITIAGGGCATTIACGGCACCTCGACCCCAAAAAACTIGAIIAGGGTGAIGGIICACGIAGTGGGCCAICGCCCTGAIAGACGGIITIII atatgtatccgctcatgagacaataaccctgataaatgcttcaataatattgaaaaagagagtatgagtattcaacatttccgtgtcgcccttattccgtgttttgcggcatttgccttcctgttt ttgctcaccagaaacgctggtgaaagtaaaagatgctgaagatcagttgggtgcacgagttacatcgaactggatctcaacagcggtaagatccttgagagttttcgccccgaagaacgttttc GCGCAACGCAATTAATGTGAGTTAGCTCACTCATTAGGCACCCCAGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGTGTGGAAATTGTGAGGGATAACAATTTCACACAGGAAACAGCTATGACCA CGCCTCTCCCCGCGCGCTTGGCCGATTCATTAATGCAGCTGGCACGACAGGTTTCCCCGACTGGAAAGCGGGCCAGTGA

Fig. 26B